EMBRYOLOGY.[1]

Ookinesis in Limax maximus.—The observations here given are confined to early stages of the egg while in the oviduct, and before the expulsion of either polar globule. The article, therefore, deals with stages which, for the most part, preceed any discussed by Dr. Mark in his excellent treatise on *L. campestris.*[2]

Of the following wood-cuts, Fig. 1 is a diagrammatic representation of the oviduct from a laying animal, from which eggs were taken, and studied serially as numbered. The vitellus averaged 156.2 μ in diameter.

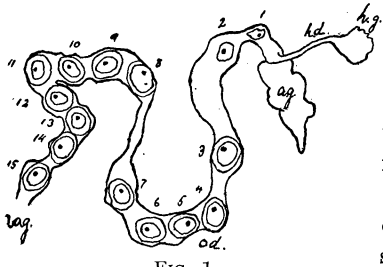

FIG. 1.

Various methods were made use of in fixing—Fols solution: osmic acid, 1 %, followed by Merkel's fluid; chromic acid, $\frac{1}{3}$%, etc., but the one which gave the best satisfaction was as follows: The body cavity of a laying animal was opened by a quick cut of the scissors, and the animal plunged into a boiling hot solution of corrosive sublimate; allowed to remain one minute; transferred to water and eggs removed from oviduct and shelled.[3] Vitellus allowed to remain in distilled water two minutes, then transferred to 35 and 50 % alcohol, remaining three minutes in each grade; then to 70 % alcohol for permanent preservation. I found that if eggs were allowed to remain in distilled water three hours or more, they shelled better, the vitellus coming out clearer and freer. For examination of eggs in toto, Czokor's alum cochineal gave, as a rule, good results. Ten minutes' stay in this dye appeared to give the necessasy differentiation; but for examination of sections much longer time was necessary, two to three hours or more. Picrocarminate of lithium was also found to be excellent, if anything, better than Czokor, on account of its differentiating nucleus structures.

[1]Edited by E. A. Andrews, Baltimore Md., to whom communications may be addressed.

[2] "The Maturation, Fecundation and Segmentation of *Limax campestris* Binney," by E. L. Mark, Bulletin of the Museum of Comparative Anatomy, Vol. 6, parts 11 and 12, Cambridge, Mass, 1881.

[3] In the upper part of the glandular portion of the oviduct there were a number of eggs in which the outer membrane or shell was barely formed, in some, egg No. 1, for example, there was no membrane at all, and in others only the inner membranous coat was present.

For examination in toto, 24 hours in this stain, and then washing with distilled water and pure alcohol gave good results.

Section staining on slide was also found desirable and Safranin was the stain used—2½ hours, followed by acidulated (½ % Hcl) alcohol of 90 % grade for 7–10 minutes.

The Schällibaum should be new, the sections carefully applied to a well smeared slide, and kept at 60° C. for exactly 15 minutes.

If Mayer's albumen fixative is used, only warm, and as soon as paraffine is melted remove slide from heat.

A number of sections of the hermaphrodite duct (h. d. Fig. 1) were made. One egg was found, in this duct, near the hermaphrodite gland, containing two polar corpuscles, each surrounded with a faintly stained Hof, and each showing striae radiating from corpuscle through Hof. About 8 chromosomes were observed irregularly grouped in the well-defined archoplasm of Boveri.[4]

From these sections it appears that the centres of attraction which Garnault[5] says do not exist in the ovarian egg of *Arion* and *Helix*, and which were not seen in the hermaphrodite gland of *L. maximus*, do exist in the duct very near the gland. They evidently appear immediately after the egg has left the ovary. This duct was lined, for the most part, with ciliated epithelium, and contained much mucus.

Fig. 2 illustrates an optical section of egg No. 1 from glandular part of the oviduct (see Fig. 1) viewed obliquely to the long axis of the spindle, and showing the two polar corpuscles and chromosomes, there being about twenty of the latter lying in an irregular cluster in the clear space between the corpuscles. This egg was stained in picrocarminate of lithium for 30 hours. In its examination a Zeiss Oc 2 and Obj. E were used. A broken membrane, "membrane rougée," was seen with apparently chromatic thickenings in it. Observations on this egg coincide closely with those of Garnault on *Arion* and *Helix*, and, in a measure, with those of Vejdovsky on *Rhynchelmis*.[6]

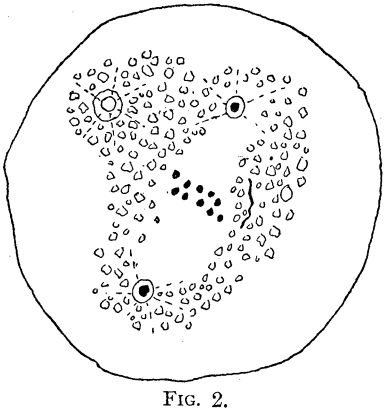

FIG. 2.

[4] "Zellen-Studen" von Dr. Theodor Boveri, Jena, 1887.

[5] "Sur les phénomènes de la fécundation chez l' Helix aspersa et l' Arion empiricorum."—Zoöl. Anzeiger Nos. 297 and 298, Dec., '88 and Jan., '89.

[6] Die Entwecklungsgeschicete der Oligochaeten (*Rhynchelmis*), 1888.

The larger corpuscle is the one nearest the observer. The structural peculiarity of one side of the nucleus should be noted—where cytoplasm and yolk granules are in intimate relation with contents of nucleus. This is Garnault's "prophase;" it is the stage just previous to formation of nuclear plate leading to the forming of first polar globule. In another egg, No. 9, from the same oviduct, an optical section showed rays of hyalocytoplasm pushing out from clear area through granules of vitellus. Chromosomes irregularly placed in hyaline area. Spindle striae observed in viewing the egg at right angles to spindle axis.

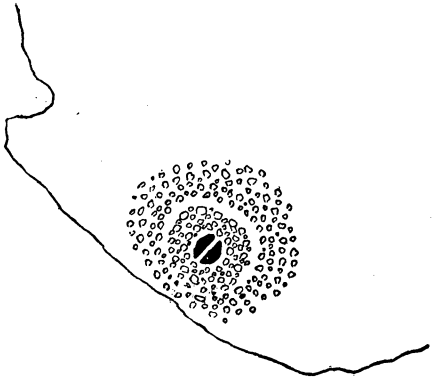

FIG. 3.

Fig. 3 illustrates an optical section of egg No. 11 from oviduct of another animal, occupying the same relative position as No. 11 in the oviduct drawn. In an eccentric position, and near the surface, a clear circular area with radial striae was observed, indicating the presence of the male pronucleus. A portion of the membrane of the germinal vesicle still present. Egg No. 10, in the same animal, also showed circular male area in direction of axis of spindle, and chromatin granules within it. In egg No. 9 the head of spermatozoön was seen in optical section, some little distance from periphery, circular with narrow Hof about it and striae radiating from Hof. Very fine granules were evident within this pronucleus.

Fig. 4 illustrates part of a section of egg shown in Fig. 2, cut in such a plane as to show the sperm nucleus near the periphery. Drawn with Zeiss Oc 1 and $\frac{1}{18}$ oil immersion. Garnault says, in speaking of formation of sperm nucles in *Arion* and *Helix*, "the spermatozoön enters just before first kinesis or immediately after. The contracted head does not begin to change until after the expulsion of the second polar globule. The sperm-head first

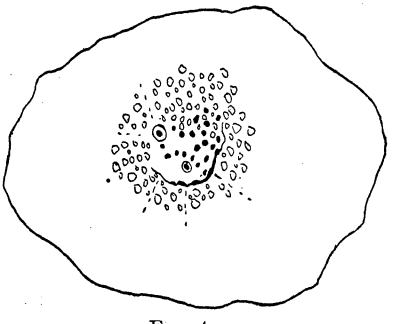

FIG. 4.

divides ihto two chromatin spherules, then, by successive divisions, there is formed a great number of spherules which remain inclosed in a clear areole. This clear areole recalls the hyaline centre of attraction when that has received the half plate for the formation of a vesicular nucleus.[7]

—F. L. WASHBURN.

[7] The following few notes pertaining to the fixing and staining of *freshly laid* eggs may be of interest.

Eggs placed for 5 minutes in Fol 99 (1 % chromic 25 vol, 2 % acetic 50 vol, H_2O 25 vol) then shelled in water, vitellus in same solution for 5 minutes, H_2O 10 min., and 35 % and 50 % alcohol 5 minutes each, 70 % 30 min. and 90 % ad. lib. gave good results, taking picrocarminate of lithium very well if left long enough in stain. They also took borax carmine very well after the above treatment.

Both of these stains did well after the eggs were immersed in chromic $\frac{1}{3}$ % 10 min., then shelled in large quantity of water, then vitellus in chromic $\frac{1}{3}$ % 4 min., and H_2O and grades of alcohol as above.

Whole egg in osmic acid 1 % 5 min., followed by Merkel's fluid 4 hrs.; shell, then water and grades of alcohol 2 min. each to 70 % for permanent preservation were quite satisfactory. It gave good results as to nuclei when eggs were left in picrocarminate of lithium for 48 hrs.